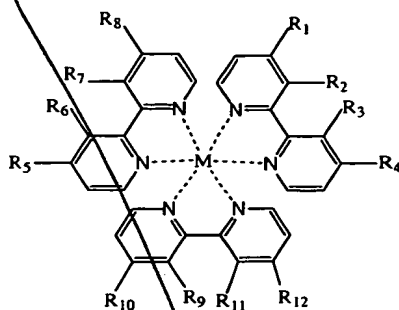


## CLAIMS

We Claim:

- 5 *Sub A1*
1. An encapsulation vesicle for an assay, comprising:
    - (a) a matrix having a surface;
    - (b) a surface coating on said matrix; *→ must be donor p. 6, 2.18*
    - (c) a protection layer encapsulating said surface coating for protecting said surface coating from a quencher molecule; and
    - (d) a ligand attached to said protection layer. *acceptor p. 8*
  - 10 2. An encapsulation vesicle as recited in claim 1, wherein said matrix is a sol gel material.
  - 15 3. An encapsulation vesicle as recited in claim 1, wherein said matrix surface comprises silica and synthetic polymer.
  - 20 4. An encapsulation vesicle as recited in claim 1, wherein the matrix surface is modified with carboxyl groups so that organometallic complexes can be covalently attached to the surface.
  - 25 5. An encapsulation vesicle as recited in claim 1, wherein the matrix surface is modified with amino groups so that organometallic complexes can be covalently attached to the surface.
  - 30 6. An encapsulation vesicle as recited in claim 3, wherein the matrix is modified so that long lifetime fluorophores can be either absorbed or covalently linked to the matrix.
  7. An encapsulation vesicle as recited in claim 1, wherein said surface coating comprises at least one donor molecule.
  8. An encapsulation vesicle as recited in claim 7, wherein said donor molecule is an organometallic material.
- Sub A2*

9. An encapsulation vesicle as recited in claim 8, wherein said donor molecule is:



where M is s selected from the group consisting of Ru, Os and Re;

R<sub>1</sub> is selected from the group consisting of H, alkyl, aryl, and aryls leading to a non-substituted or substituted phenanthroline;

R<sub>2</sub> is selected from the group consisting of H, alkyl, aryl, and aryls leading to a non-substituted or substituted phenanthroline;

R<sub>3</sub> is selected from the group consisting of H, alkyl, aryl, and aryls leading to a non-substituted or substituted phenanthroline;

R<sub>4</sub> is selected from the group consisting of H, alkyl, aryl, and aryls leading to a non-substituted or substituted phenanthroline;

R<sub>5</sub> is selected from the group consisting of H, alkyl, aryl, and aryls leading to a non-substituted or substituted phenanthroline;

R<sub>6</sub> is selected from the group consisting of H, alkyl, aryl, and aryls leading to a non-substituted or substituted phenanthroline;

R<sub>7</sub> is selected from the group consisting of H, alkyl, aryl, and aryls leading to a non-substituted or substituted phenanthroline;

R<sub>8</sub> is selected from the group consisting of H, alkyl, aryl, and aryls leading to a non-substituted or substituted phenanthroline;

R<sub>9</sub> is selected from the group consisting of H, alkyl, aryl, and aryls leading to a non-substituted or substituted phenanthroline;

R<sub>10</sub> is selected from the group consisting of H, alkyl, aryl, and aryls leading to a non-substituted or substituted phenanthroline;

~~R<sub>11</sub> is selected from the group consisting of H, alkyl, aryl, and aryls leading to a non-substituted or substituted phenanthroline; and~~

~~R<sub>12</sub> is selected from the group consisting of H, alkyl, aryl, and aryls leading to a non-substituted or substituted phenanthroline.~~

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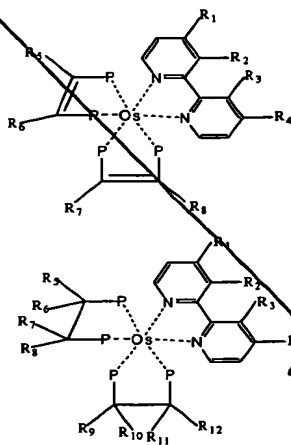
Sub A3

~~10. An encapsulation vesicle as recited in claim 8, wherein said organometallic material is a ruthenium tris diphenyl phenanthroline complex.~~

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~~11. An encapsulation vesicle as recited in claim 8, wherein said organometallic material has an emission at about 650 nm.~~

~~12. An encapsulation vesicle as recited in claim 8, wherein the donor molecule is selected from the group consisting of:~~



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~~where R<sub>1</sub>, R<sub>2</sub>, R<sub>3</sub>, R<sub>4</sub>, represent H, alkyl, aryl, aryl leading to the formation of non-substituted or substituted phenanthroline; and~~

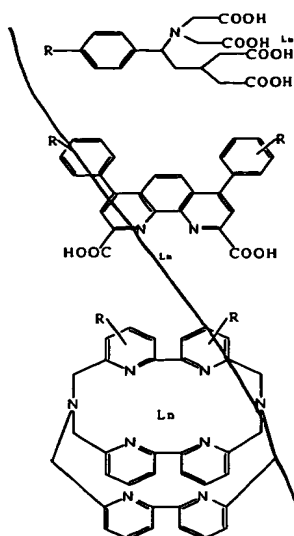
20

~~wherein R<sub>5</sub>, R<sub>6</sub>, R<sub>7</sub>, R<sub>8</sub>, R<sub>9</sub>, R<sub>10</sub>, R<sub>11</sub>, R<sub>12</sub> represent H, alkyl, aryl, aryl leading to the formation of ortho-aromatic phosphines.~~

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Sub A4

~~13. An encapsulation vesicle as recited in claim 8, wherein said donor molecule is selected from the group consisting of:~~



where  $L_n = \text{Eu, Tb, Sm, Dy}$ , and  $R$  represents  $\text{H}$  or a functionality capable of covalently linking to a matrix .

14. An encapsulation vesicle as recited in claim 8, wherein the donor molecule is any molecule having a fluorescence lifetime greater than 100 nanoseconds and the molecules are susceptible to quenching by oxygen.

15. An encapsulation vesicle as recited in claim 2, wherein said protection layer comprises a translucent material.

16. An encapsulation vesicle as recited in claim 2, wherein said protection layer comprises a transparent material.

17. An encapsulation vesicle as recited in claim 2, wherein said protection layer comprises sol-gel (silica) and synthetic polymers.

18. An encapsulation vesicle as recited in claim 2, wherein said protection layer is modified with hydrophilic functionalities selected from the group consisting of hydroxyl, carboxyl and protonated amines.

19. An encapsulation vesicle as recited in claim 2 that was formed by suspension polymerization.

*Sub A5*  
~~20. An encapsulation vesicle as recited in claim 1, wherein said ligand attached to said protection layer further comprises an acceptor molecule capable of receiving energy transfer from said donor molecule of said surface coating.~~

21. An encapsulation vesicle as recited in claim 1, wherein said assay is an immunoassay.

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~~22. An encapsulation vesicle as recited in claim 3, further comprising a ligand attached to the protection layer and having an acceptor molecule capable of receiving energy transfer from a donor molecule.~~ *already in claim 1*

*Sub A6*  
15  
~~23. An encapsulation vesicle as recited in claim 1, wherein the acceptor's absorption band overlaps with the emission band of the donor.~~ *where?*

24. An encapsulation vesicle as recited in claim 1, wherein the acceptor is selected from the group consisting of fluorescein, Cy5 and allophycocyanin. *no donor present*

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25. An encapsulation vesicle as recited in claim 1, wherein said ligand is an antibody.

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26. An encapsulation vesicle as recited in claim 1, wherein said assay is a sandwich assay.

30  
27. An encapsulation vesicle as recited in claim 1, wherein the biomolecule is selected from the group consisting of proteins, DNA, RNA, polypeptides, aptamers and receptor molecules.

~~28. A method of quantifying an analyte in a sample, comprising the steps of:  
(a) mixing a first binding molecule with a second binding molecule, wherein the first binding molecule competes with an analyte for binding the second binding molecule, wherein one of the first and~~

second binding molecules is labeled with a photoluminescent energy transfer donor and the other is labeled with a photoluminescent energy transfer acceptor, wherein the photoluminescent energy transfer donor and acceptor are chosen such that when the first binding molecule binds to the second binding molecule, the donor and acceptor are brought into interacting proximity, producing a detectable luminescence change in the donor;

- (b) encapsulating a second binding molecule;
- (c) exposing the sample to an exciting amount of radiation;
- (d) detecting the resulting emission; and
- (e) calculating the apparent luminescence of the donor to quantify binding of the first binding molecule to the second binding molecule and thereby inversely quantifying the analyte.

29. The method of claim 29, wherein the photoluminescent donor is selected from the group consisting of cyanines, oxazines, thiazines, porphyrins, phthalocyanines, fluorescent infrared-emitting polynuclear aromatic hydrocarbons, phycobiliproteins, squaraines and organo-metallic complexes.
30. The method of claim 29, where the photoluminescent acceptor is selected from the group consisting of cyanines, oxazines, thiazines, porphyrins, phthalocyanines, polynuclear aromatic hydrocarbons, phycobiliproteins, squaraines, organo-metallic complexes, and azo dyes.
31. The sandwich method of quantifying an analyte in a sample, comprising the steps of:

- (a) mixing a first binding molecule with a second binding molecule, wherein the first binding molecule competes with an analyte for binding the second binding molecule, wherein one of the first and second binding molecules is labeled with a photoluminescent energy transfer donor and the other is labeled with a photoluminescent energy transfer acceptor, wherein the photoluminescent energy transfer donor and acceptor are chosen such that when the first binding molecule binds to the second binding molecule, the donor and acceptor are

$\Delta$  from 28

- (b) encapsulating a second binding molecule;
- (c) exposing the sample to an exciting amount of radiation;
- (d) detecting the resulting emission; and
- (e) calculating the apparent luminescence lifetime of the donor without the use of a fluorescence intensity measurement to quantify the immune complex, thereby quantifying the analyte.

add A7

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